ELISA PROTOCOL

MATERIALS

1 96 well plate
100 ml TBS-T
10 ml PBS-B (1% BSA)
10 ml PBS
1.7 ml HER2, 1 ug/ml (17 ul stock + 1683 ul PBS-B 1% BSA)
1.7 ml HER2, 3 ug/ml (51 ul stock + 1649 ul PBS-B 1% BSA)
1.7 ml HER2, 5 ug/ml (85 ul stock + 1615 ul PBS-B 1% BSA)
2.5 ml Anti-Affi, 1:2500 in PBS-B (1% BSA)
2.5 ml Anti-goat, 1:2500 in PBS-B (1% BSA)
2.5 ml Anti-V5, 1:2500 in PBS-B (1% BSA)
2.5 ml Anti-mouse, 1:2500 in PBS-B (1% BSA)
1.3 ml Cell lysate, Hi concentration: 25 ul, diluted to 1300 ul in PBS-B (1% BSA)
1.3 ml Cell lysate, Low concentration: 12.5 ul, diluted to 1300 ul in PBS-B (1% BSA)
1.3 ml Negative control diluted to 1300 ul in PBS-B (1% BSA)
650 ul Z_{HER2} diluted 1:2000 to a final concentration of 0.5 ug/ml in PBS-B (1% BSA)
650 ul V5 positive control diluted to 650 ul in PBS-B (1% BSA)
5 ml TMB substrate
5 ml 2M H_2SO_4

METHOD

1. Coat half the plate with three different concentrations of HER2 (in PBS-B, 1%), 100 ul/well, according to the loading pattern and incubate overnight at 4 °C (cold room). Cover the plate with a dedicated adhesive plastic film and mark the film rather then the plate.
2. Block the plate with 200 ul/well of PBS-B, 1%. Cover the plate with the dedicated adhesive plastic film and incubate at room temperature and shake for 2h.
3. Load 100 ul/well of samples according to the loading pattern. Cover the plate with the dedicated adhesive plastic film and incubate in 37 °C, 150 rpm for 1h 30min.
4. Wash with 200 ul/well TBS-T in RT and shake for 3-5 minutes. Repeat in total three times.
5. Load 100 ul/well with the first antibody, diluted in PBS-B according to the loading pattern. Cover the plate with the dedicated adhesive plastic film and incubate in RT and shake for 1h. Make sure that the antibodies are diluted to the correct concentration as shortly as possible before loading!
6. Wash with 200 ul/well TBS-T in RT and shake for 3-5 minutes. Repeat in total three times.
7. Load 100 ul/well with the secondary antibody (with HRP), diluted in PBS-B according to the loading pattern. Cover the plate with the dedicated adhesive plastic film and incubate in RT and shake for 1h. Make sure that the antibodies are diluted to the correct concentration as shortly as possible before loading!
8. Wash with 200 ul/well TBS-T in RT and shake for 3-5 minutes. Repeat in total four times. Do one final wash with 200 ul/well PBS in RT and shake for 3-5 minutes.

9. Develop the plate with 100 ul/well TMB substrate for 15-30 minutes until the color change is stabilizing. Stop the reaction with 100 ul/well 2M H_2SO_4 and the color will change from blue to yellow.

Figure 1. ELISA loading pattern

HER2 conc.	**1 ug/ml**		**3 ug/ml**		**5 ug/ml**		
$Sample_{Hi}$	A 1	2	3	4	5	6	Anti-Affi
$Sample_{Low}$	B						Anti-goat
(+) Z_{HER2}	C						
(-)	D						
$Sample_{Hi}$	E						Anti-V5
$Sample_{Low}$	F						Anti-mouse
(+)	G						
(-)	H						

TIPS:

The plate can be found in the storage room on the right side.

Use a multi-pipette for the washing steps and development (and other steps if you want). Trays can be found to the right in the storage room.

TBS-T and H_2SO_4 was borrowed from Anna-Louisa and can be found in the cupboard above her lab space.

BSA can be found in the common fridge.

Z_{HER2} can be found diluted 1:500 (if I remember correctly) in the recognition box in the freezer.

To empty the wells just throw it out the sink and beat the plate upside down a couple of times on some paper towel.

TMB substrate is in a box in the common fridge. It consists of two bottles which are to be mixed directly before usage. Keep these on the bench a couple of hours before use as they work better in RT then cold.

The wells will rely on surface tension when loaded with 200 ul (washing steps). This shouldn't be a problem.

Just give me a call or send me a text if you have any questions!